# Biotechnology of Specialty Fats

**Purwiyatno Hariyadi**
SEAFAST Center/
Dept. Ilmu & Teknologi Pangan,
INSTITUT PERTANIAN BOGOR
www.seafast.ipb.ac.id

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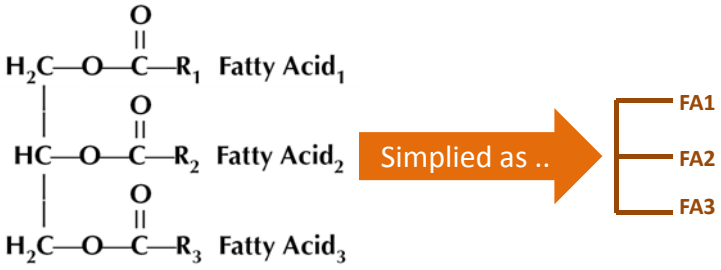
JAKARTA, 27-28 JUNI 2013

## Introduction

Natural oils and fats: Specific distribution of fatty acids (FAs) in its triacyl glycerols (TAGs)



# Introduction

## Fatty acid composition of different types of edible oil

| Fatty acids % | Sunflower oil (n=5) Mean ± SD | Soybean oil (n=3) Mean ± SD | Mustard oil (n=5) Mean ± SD | Palm oil (n=3) Mean ± SD | Coconut oil (n=6) Mean ± SD |
|---|---|---|---|---|---|
| Caprylic ($C_{8:0}$) | -- | -- | -- | -- | 6.21 ± 0.34 |
| Capric ($C_{10:0}$) | -- | -- | -- | -- | 6.15 ± 0.21 |
| Lauric ($C_{12:0}$) | -- | -- | -- | -- | 51.02 ± 0.71 |
| Myristic ($C_{14:0}$) | -- | -- | -- | 1.23 ± 0.28 | 18.94 ± 0.63 |
| Palmitic ($C_{16:0}$) | 6.52 ± 1.75 | 14.04 ± 0.62 | 4.51 ± 3.83 | 41.78 ± 1.27 | 8.62 ± 0.50 |
| Stearic ($C_{18:0}$) | 1.98 ± 1.44 | 4.07 ± 0.29 | 2.78 ± 0.59 | 3.39± 0.65 | 1.94 ± 0.17 |
| Oleic ($C_{18:1}$) | 45.39 ± 18.77 | 23.27 ± 2.43 | 38.21 ± 21.88 | 41.90 ± 1.20 | 5.84 ± 0.50 |
| Linoleic ($C_{18:2}$) | 46.02 ± 16.75 | 52.18 ± 2.64 | 25.31 ± 5.74 | 11.03 ± .02 | 1.28 ± 0.18 |
| Linolenic ($C_{18:3}$) | 0.12 ± 0.09 | 5.63 ± 3.48 | 11.30 ± 6.09 | -- | -- |
| Arachidic ($C_{20:0}$) | -- | -- | 10.86 ± 3.29 | -- | -- |
| Erucic ($C_{22:1}$) | -- | -- | 11.35 ± 13.83 | -- | -- |

K. Chowdhury, L. A. Banu, S. Khan and A. Latif, 2007



# Introduction

## Fatty acid composition of different types of edible oil

Red Fruit (*Pandanus conoideus*) Oil?

| Fatty Acids | Composition (%) |
|---|---|
| C10:10 | 0.01 |
| C12:0 | 0.25 |
| C14:0 | 0.14 |
| C15:0 | 0.20 |
| C16:0 | 23.70 |
| C18:0 | 0.72 |
| C18:1 | 79.50 |
| C18:2 | 4.50 |
| C18:3 | 3.20 |
| C20:1 | 0.16 |

**Andarwulan, N., Palupi, N.S and Susanti (2010)** Pengembangan Metode Ekstraksi dan Karakterisasi Ekstrak Buah Merah (*Pandanus conoideus* Lam.)

## Introduction : Fat Modification?

Natural or native fats and oils are not always appropriate for the specific application and/or consumers' demands.

Lipid Modification

Fats with the right balance of physical, chemical and nutritional characteristics; **suitable for specific application**

**= Specialty Fats**

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## (Bio)technology of Specialty Fats?

(Bio)technology of specialty fats is a fat modification technology for a specific objective, especially to obtain :

- Fats with specific **physical characteristics**
- Fats with specific **chemical characteristics**
- Fats with specific **nutritional characteristics**

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## (Bio)technology of Specialty Fats?

OVERALL ..(bio)technology of specialty fats may have a specific objective to:

- Improve oxidative stability
- Alter the melting properties
- Alter the crystallization properties
- Improve nutritional properties
- Improve health functionality



## (Bio)technology of Specialty Fats?

OVERALL ..(bio)technology of specialty fats may have a specific objective to:

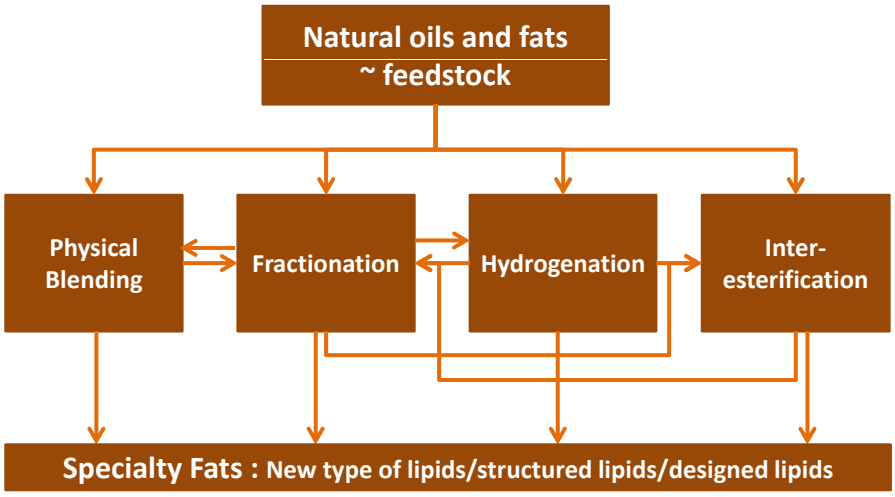
- Produce **special** of specialty fats : **structured lipid**

  → Structured lipids (often called as designed lipids) are lipids specifically designed to have special molecular structure to performe special applications :

  (1) medical applications (functional foods), and

  (2) food application

## Type of (Bio)technology of Specialty Fats?

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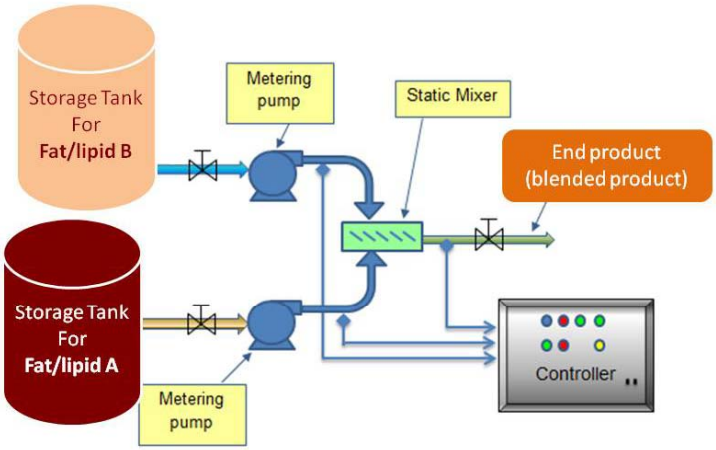
## A. Blending

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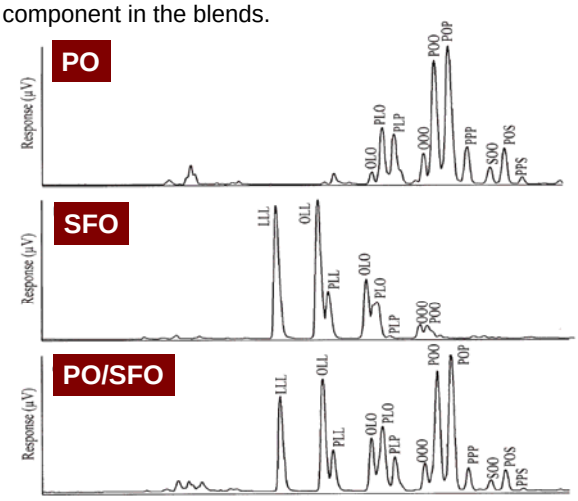
## A. Blending → It will not change chemical structure of the fat molecule

TAG composition of the blended fat represents a linear combination of the fat component in the blends.




TAG gas chromatograms of palm oil (PO), sunflower oil (SFO), and the blends of PO/SFO in the ratio of 1:1.

O, oleic acid; P, palmitic acid; L, linoleic acid; S, stearic acid.

Noor Lida, H.M.D. , Sundram, K., Siew, W.L., Aminah, A. and Mamot, S. 2002. TAG Composition and Solid Fat Content of Palm Oil, Sunflower Oil, and Palm Kernel Olein Blends Before and After Chemical Interesterification. JAOCS, Vol. 79, no. 11. p 1137-1144.

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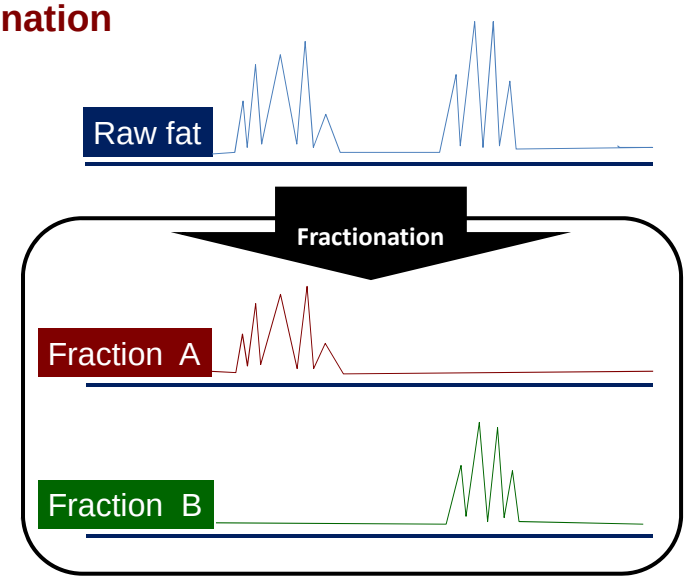
## B. Fractionation

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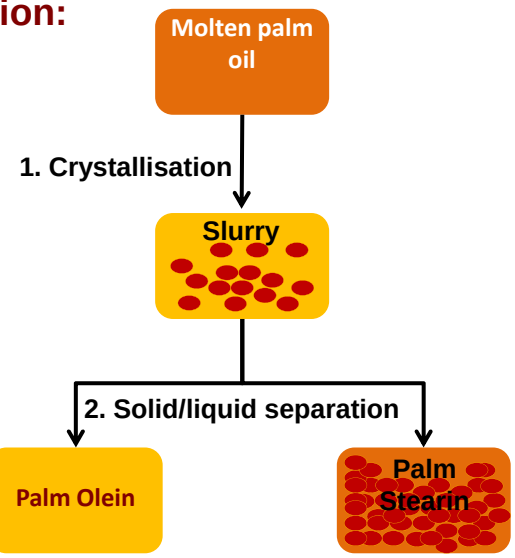
## B. Fractionation:
Principles




Modified from

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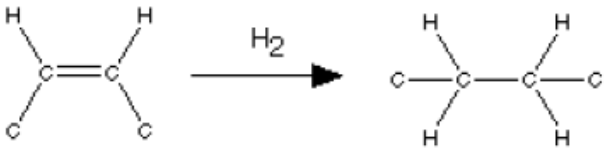
## C. HYDROGENATION

Hydrogenation, addition of hydrogens to the double bonds of unsaturated fatty acids.




Hydrogenation process :

1. Saturation of double bonds
2. Migration of double bonds, and
3. Trans-fatty acid formation

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## C. HYDROGENATION

Hydrogenation has two purposes.

1. To impart oxidative stability to the oil.
2. To convert converts the liquid oil into a semi-solid or solid fat.

A typical example of hydrogenation is in the process of margarine and shortening production.

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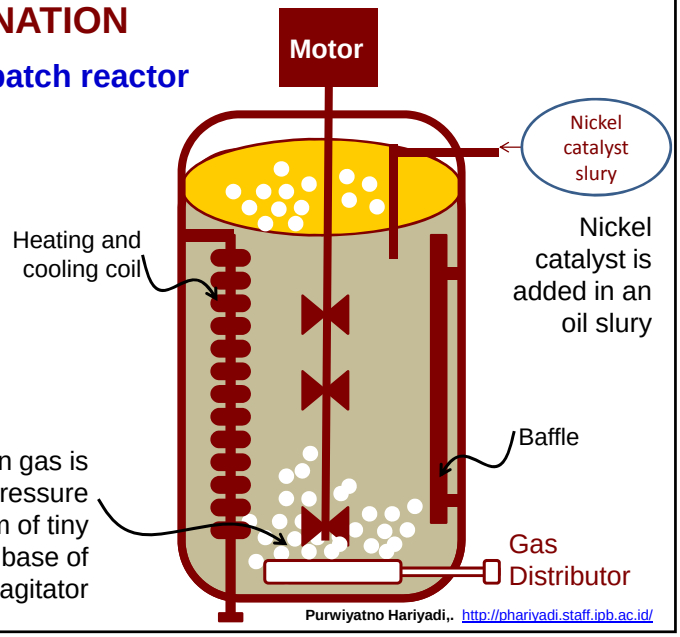
## C. HYDROGENATION

**Stirred-slurry-batch reactor**

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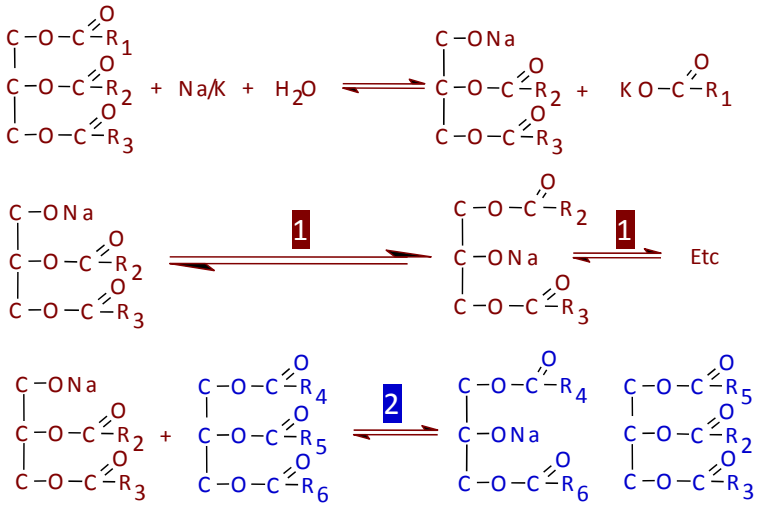
## D. INTERESTERIFICATION

- Interesterification is an acyl-arrangement reaction.
  - Distribution of FAs is randomized on the glycerol backbone.
    - Altered triacylglycerol composition
    - Increased triacylglycerol species.
      - Affect the physical characteristics of the oil or fat, including melting and crystallization.

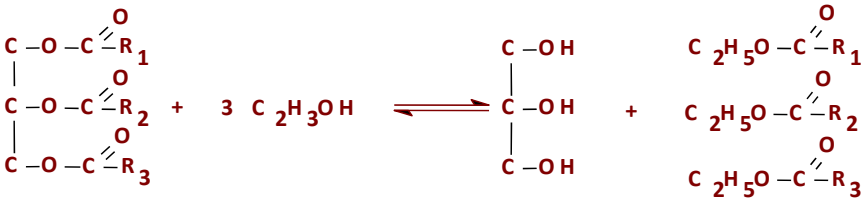


## Type of Interesterification (1)

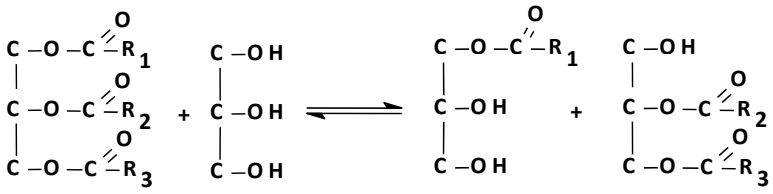



**Ester exchange/interchange :**
**(1) Intramolecular rearrangement, (2) Intermolecular rearrangement**

## Type of Interesterification (2)



**Alcoholysis reactions**



**Glycerolysis reactions**

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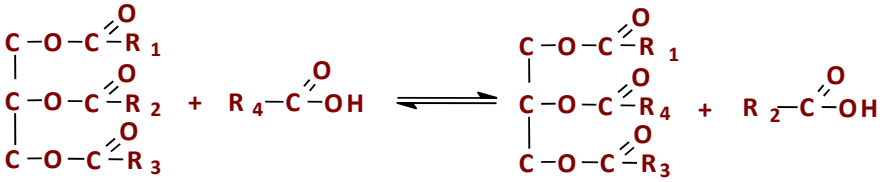
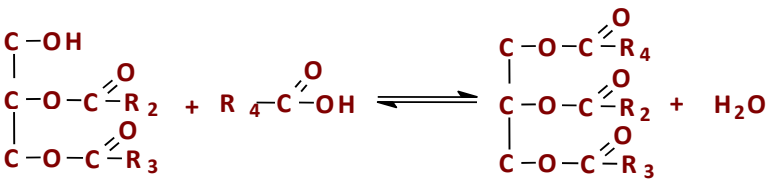
## Type of Interesterification (3)





**Acidolysis reactions**

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→ INTERESTERIFICATION is a much more powerful tool than blending of different oils or fats.

→ Interesterification vs. Hydrogenation (margarine and spread production)



The triacylglycerols composition of natural and interesterified milk fat

| Triacylglycerols (TAGs) | Native | Interesterified |
|---|---|---|
| C22 | - | 0.1 |
| C24 | 0.3 | 0.8 |
| C26 | 0.1 | 1.4 |
| C28 | 0.6 | 1.5 |
| C30 | 0.9 | 1.3 |
| C32 | 1.9 | 2.0 |
| C34 | 4.4 | 3.0 |
| C36 | 9.5 | 5.9 |
| C38 | 13.1 | 9.1 |
| C40 | 12.1 | 9.9 |
| C42 | 7.7 | 7.6 |
| C44 | 6.8 | 8.3 |
| C46 | 7.5 | 10.7 |
| C48 | 8.8 | 12.8 |
| C50 | 11.2 | 14.2 |
| C52 | 10.8 | 10.9 |
| C54 | 4.6 | 0.4 |

## Interesterification Changes Melting Point of fats

| Fat | Melting Point (°F) Before IE | After IE |
|---|---|---|
| *Soybean oil* | *19.4* | *41,9* |
| Cottonseed oil | 50.9 | 93.2 |
| *Coconut oil* | *78.8* | *82.8* |
| Palm oil | 10.7 | 116.6 |
| *Lard* | *109.4* | *109.4* |
| Tallow | 115.2 | 112.3 |
| *40% hydrog. Cottonseed oil + 60% coconut oil* | *136.0* | *106.0* |
| 75% soybean oil + 25% tristearin | 140.0 | 90.0 |
| *25% hydrog. PO + 75% hydrog. PKO* | *122.3* | *104.5* |

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## ... SPESIFIC OBJECTIVES OF INTERESTERIFICATION (IE)

- Production of triglycerides with specific fatty acid composition and stereospecific distribution, i.e. tailor made fats and oils = structured lipids.
- Production of dietetic fats, i.e. fats are upgraded by the incorporation of polyunsaturated/essential fatty acids.
- Production of high value fats : CBS, CBE, CBX
- Production of specific material :
  Intramolecular esterification = polymerization

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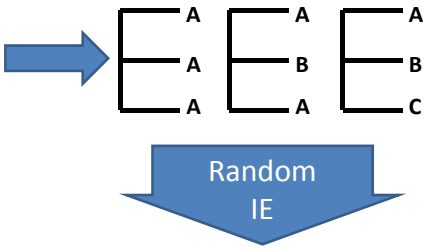
Two important types of Interesterification (IE) are used :

- Random interesterification
- Directed interesterification



## 1. Random Interesterification (1)

Triacylglycerol (TAG) molecules in fats and oils can be either made up of a single fatty acid (FA) species or any combination of up to three different FAs

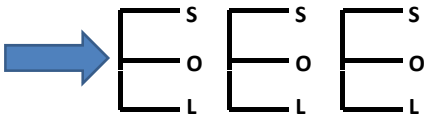



$\%\ AAA = a^3/10000$
$\%\ AAB = 3a^2b/10000$
$\%\ ABC = 6abc/10000$

*a, b* and *c* are the concentrations of fatty acids A, B and C (mol. %).
AAA, AAB and ABC are mono-, di-, and tri acid TAG, respectively.

## 1. Random Interesterification (2)

Case of
1-stearoyl-2-oleoyl-3-linoleoyl glycerol




Random IE

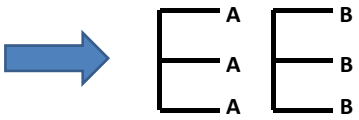
| | | | |
|---|---|---|---|
| SSS | = 3.7% | SOO | = 11. 1% |
| OOO | = 3.7% | SLL | = 11.1 % |
| LLL | = 3.7% | OOL | = 11.1% |
| SSO | = 11.1% | OLL | = 11.1% |
| SSL | = 11.1% | SOL | = 22.2% |

S = stearic, O = oleic, and L = linoleic

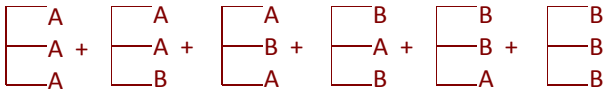


## 1. Random Interesterification (3)

Case of Interesterification of a binary mixture (50/50) of AAA and BBB triacylglycerols :




Random IE




| | | | | | | |
|---|---|---|---|---|---|---|
| Eq. composition : | 12,5% | 25% | 12,5% | 12,5% | 25% | 12,5% |

## 2. Directed Interesterification (1)

Directed IE:

IE reaction directed away from its usually random end-point, directed toward a specific fats or oils of interest.

- Physical method, and
- Enzymatic methods.



## 2. Directed Interesterification (1)

**Physical methods :**

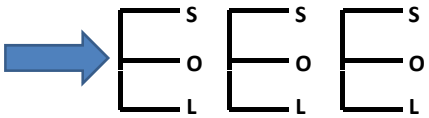
- Allowing a certain fats/oils to crystallize during reactions
- Removing part of crystalyzed triglycerides from the reaction mixture
- Allowing the IE reaction to continue at liquid phase

→ Thus selective crystallization of a fat or mixed fats may be directed to the effective conversion of all the saturated fatty acids to trisaturated triglycerides.

## 2. Directed Interesterification (1)

### Physical methods :

Case of
1-stearoyl-2-oleoyl-3-linoleoyl glycerol




Directed IE

| | | |
|---|---|---|
| Solids | SSS | 33.33 mol % |
| Liquids | OOO | 8.33 mol % |
| | LLL | 8.33 mol % |
| | OOL | 24.99 mol % |
| | OLL | 24.99 mol % |



## 2. Directed Interesterification (1)

May occur without the aid of a catalyst, but

1. Very high temperature (300$^{o}$C) is required
2. Reaction proceeds to equilibrium very slowly (long period of reaction is required)
   - Triglycerides undergo some decomposition and polymerization, and there will be development of free fatty acids.

## 2. Directed Interesterification (2) … Chemical Catalysis

**CATALYSTS**

Numerous compounds have been identified as catalyst for Interesterification at lower temperature (see Table in the next Slide).



## 2. Directed Interesterification (2) … Chemical Catalysis

| CATALYSTS | Level (%) | Temp. (°C) | Time |
|---|---|---|---|
| Metal salts<br>Acetates, carbonates, chlorides, nitrates, oxides of Sn, Zn, Fe, Co and Pb | 0.1-2 | 120-260 | 0.5-6 h under vacuum |
| Alkali hydroxides<br>NaOH, KOH, LiOH | 0.5-2 | 250 | 1.5 h under vacuum |
| Alkali hydroxide + glycerol | 0.05-0.1<br>0.1-0.2 | 60-160 | 30-45 min under vacuum |
| Metal soaps<br>Sodium stearate | 0.5-1 | 250 | 1 h under vacuum |
| Glyceride<br>Li Al stearate<br>Na Ti stearate | 0.2 | 250 | 1 h under vacuum |
| Alkali metals<br>Na, K, Na/K alloy | 0.1-1 | 25-270 | 3-120 min |
| Metal alkylates<br>Sodium methylate, ethylate, t-butylate, etc. | 0.2-2 | 50-120 | 5-120 min |
| Metal hydrides<br>Sodium hydride | 0.2-2 | 170 | 3-120 min |
| Metal amide<br>Sodium amide | 0.1-1.2 | 80-120 | 10-60 min |

## 2. Directed Interesterification (3) … Enzymatic

**Enzymes have advantages for industrial processing.**

- Mild conditions
- Reduced waste.
- High specificity :
  - directed reaction (specific products produced)
  - increase yield by reducing unintended reactions.

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## 2. Directed Interesterification (3) … Enzymatic

**…… (1) Lipase Specificity ?**

| | |
|---|---|
| **I. Substrate :** | **a) Different rates of lipolysis of TAG, DAG and MAG by the same enzyme**<br>**b) Different rates of esterification of TAG, DAG and MAG by the same enzyme** |
| **II. Positional:** | **a) Primary esters,**<br>**b) Secondary esters and**<br>**c) All three esters, non-specific or random hydrolysis** |
| **III. Fatty acid:** | **Preference for fatty acids, e.g. short chain, etc.** |
| **IV. Stereospecificity:** | **Faster hydrolysis of one primary sn ester as compared to the other.** |
| **V. Combinations of I-IV** | |

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## 2. Directed Interesterification (3) … Enzymatic

### ……. (2) Lipase Specificity ?

| | Type of specificity | Source of lipase | Reaction |
|---|---|---|---|
| I. | Substrate | | |
| | a. Different acylglycerols: Same lipase | Pancreas | TG>DG>MG<br>16:0-16:0-4:0>16:0-4:0-4.0 |
| | b. Different lipases: for TG, DG and MG | Postheparin plasma (lipoprotein lipase) | DG →MG → glycerol |
| II. | Positional | | |
| | a. Primary esters | Pancreas | TG →1,2(2,3)DG → 2-MG |
| | b. Secondary esters | *Geotrichum candidum* | Acid must contain cis-9-double bond |
| | c. All esters | *Candida cylindracea* | TG →1,2+2,3+1,3DG →1- +2-MG |
| III. | Fatty Acid | | |
| | a. 4:0-10:0 | Pregastric esterase | 4:0-10:0>12:0-18:0,18:1 |
| | b. *cis*-9-unsaturation | G. *candidum* | Acid must contain *cis*-9- double bond |
| | c. 8:0-12.0 | Rat and human lingual preparations | 8:0-12:0>16:0, 18:1 |

R-long chain fatty acids in human milk



## 2. Directed Interesterification (3) … Enzymatic

### ……. (3) Lipase Specificity ?

| | Type of specificity | Source of lipase | Reaction |
|---|---|---|---|
| IV. | Stereospecificity | | |
| | a. Sn-1-ester | Postheparin plasma, bovine and human milks, adipose tissue, liver (lipoprotein lipase) | sn-1:sn-3,2:1 |
| | b. Sn-3-ester | Rat and human lingual preparations; termite | sn-3:sn:1,4:1 |
| V. | Combinations | | |
| | a. Fatty acid and stereospecificities | Rat lingual preparations | Sn-R-R$^a$-12:0>sn-R-R-16:0 |
| | | Human milk lipoprotein lipase | 8:0, 10:0, 12:0 and 18:0<br>TG>16:0-16:0-16:0. sn-1:sn-3,2:1 |

R-long chain fatty acids in human milk

## 2. Directed Interesterification (4) … Enzymatic in Organic Solvent

**Suitability of solvents for supporting enzymatic esterification reaction**

**Acetonitrile (Log P : -0,33)**
**Acetone (Log P : -0,23)**
**Tetrahydrofuran (Log P : 0,49)**
**Chloroform (Log P : 2,0)**

→ **Not supportive for esterification reaction**

**Benzene (Log P : 2,0)**
**Toluene (Log P : 2,5)**
**Hexane (Log P : 3,5)**
**Decane (Log P : 5,6)**

→ **supportive for esterification reaction**

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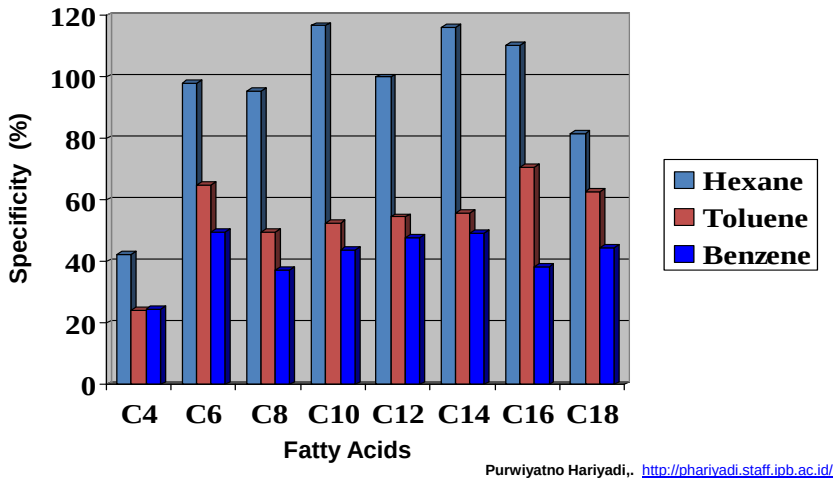
## 2. Directed Interesterification (4) … Enzymatic in Organic Solvent

***C. antartica*** **Lipase specificity toward fatty acids of various chain length**
**Specificity was measured for esterification synthesis of octyl ester**
**(Specificity of Dodecanoic acid was taken as 100%)**

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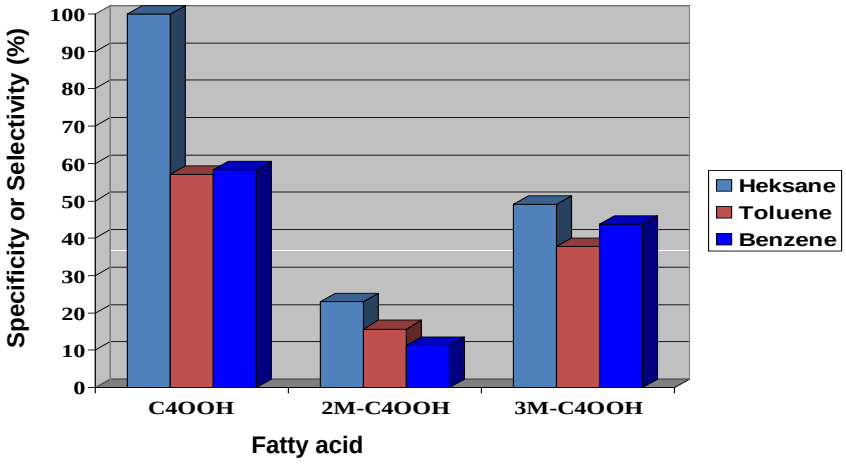
## 2. Directed Interesterification (4) … Enzymatic in Organic Solvent

**Butyric acid specificity of *C. antartica* lipase as affected by the presence of methyl group**

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## 2. Directed Interesterification (4) … Enzymatic in Organic Solvent

$CH_3—CH_2—CH_2—COOH$ **Butanoic (butyric) acid**

$CH_3—CH_2—CH(CH_3)—COOH$ **2-metilbutanoic acid**

$CH_3—CH(CH_3)—CH_2—COOH$ **3-metilbutanoic acid**

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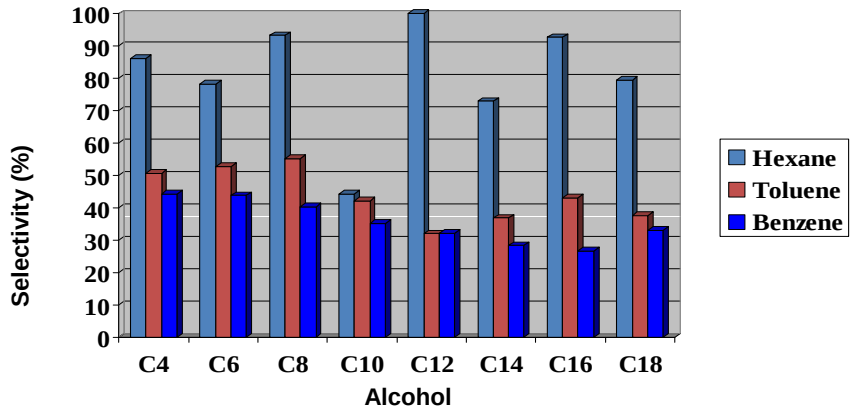
## 2. Directed Interesterification (4) … Enzymatic in Organic Solvent

**Esterification specificity or selectivity of *C. antartica* lipase toward fatty acohol as measured for esterification synthesis of dekanoic alkyl ester (in hexane), hexadecanoic alkyl ester (in toluene) and hexanoic alkyl ester (in benzene)**

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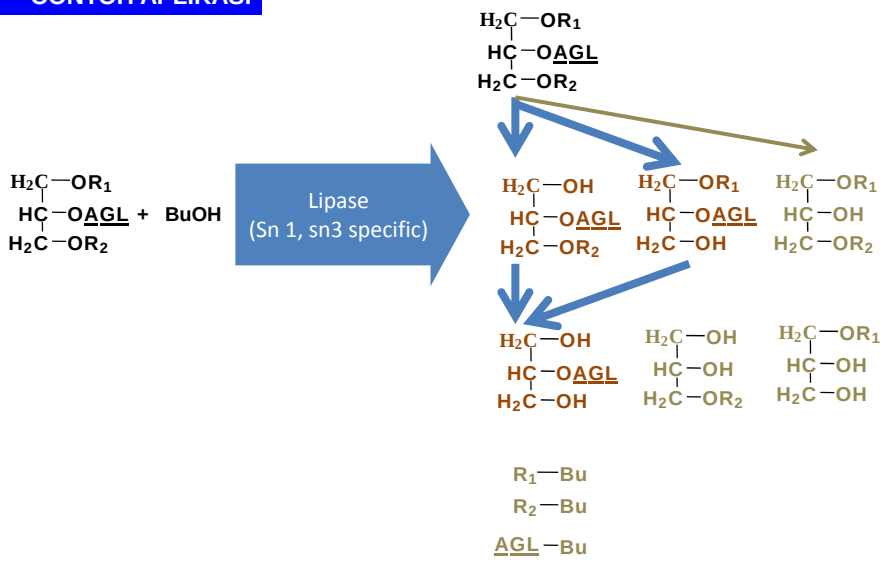
## CONTOH APLIKASI

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## CONTOH APLIKASI

Minyak kapang

↓

Alkoholisis (heksan, lipase, BuOH)

↓

Camp : Butil ester, MAG, DAG, TAG

↓

destilasi → Butil ester

↓

camp. Asilgliserol kaya AGL

↓

fraksinasi

↓

MAG kaya AGL (komponen fungsional ganda)

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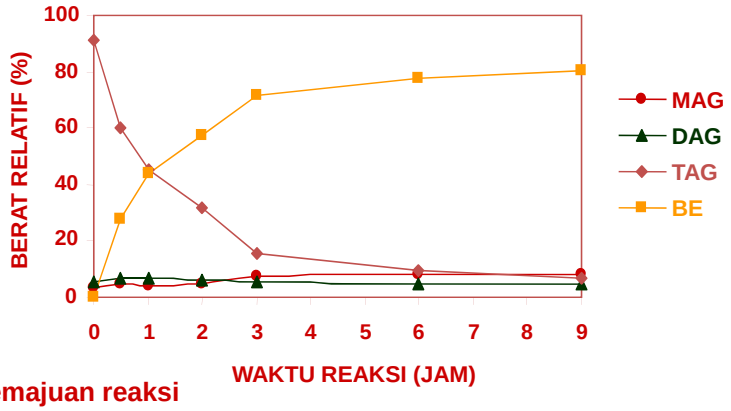
## CONTOH APLIKASI




**Kurva kemajuan reaksi**

***Kondisi reaksi alkoholisis adalah : 200 mg minyak kapang (0,2334 mmol) dengan 69,08 mg n-butanol (0,9335 mmol) dalam 2 ml heksana, menggunakan katalis lipozyme 13,454 mg (5% dari total berat substrat) pada suhu 50$^{o}$C. Data adalah hasil dari dua kali ulangan.***

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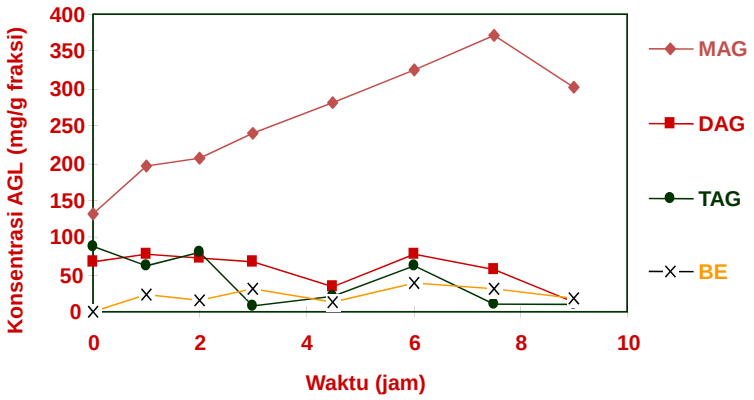
## CONTOH APLIKASI




**Konsentrasi AGL pada fraksi MAG, DAG, TAG, dan BE selama reaksi alkoholisis pada suhu 50ºC.**



## CONTOH APLIKASI

KONSENTRAT AGL DARI MINYAK KAPANG : PROSES "OPTIMUM"

| | | |
|---|---|---|
| Enzim | : | Lipozyme (*R. miehei*) (kadar air: 9,65%, $a_w$ : 0,42, pH :4,0) |
| Konsentrasi enzim | : | 2% (w/w) |
| Alkohol | : | n-butanol |
| Nisbah Molar | : | minyak kapang/BuOH (1:1) |
| Suhu | : | 50 C |
| Waktu | : | 6 jam |
| Konsentrat | : | |
| Camp asilgliserol | : | 31,82%(w/w) |
| Kandungan AGL | : | 205,72 mg/g (pemekatan 2,5 kali)* |
| Monoasilgliserol (MAG) | : | 11,45%(w/w) |
| kandungan AGL | : | 324,60% mg/g (pemekatan 3,9 kali) |

* kandungan AGL pada minyak kapang = 82,32 mg/g

## Example of Specialty Fats? ...(1)

Neobee SL220™ (produced by Stepan Co., Maywood, NJ):
Structured lipids containing n-6 and n-3 FA as well as a small amount of medium-chain FA

Neobee 1827™ (Stepan Co., Maywood, NJ):
structured lipids containing medium-chain FA and fatty acid from sunflower oil



## Example of Specialty Fats? ...(2)

Benefat™ (Cultor Food Science Inc., NY) ; Salatrim.

Salatrim (originally developed by Nabisco, Inc.) = ***S**hort **a**nd **l**ong **a**cyl**tri**glyceride **m**olecule)* is a family of TAG constituting mixtures of long- and short-chain saturated FA randomly esterified to glycerol.

Low-cal fats : 5 Kcal/g (vs. 9 Kcal/g for typical fats)

Short-chain FA used are acetic, propionic, and butyric acids, long-chain FA is stearic acid

Used in chocolate confectionery products & baked goods

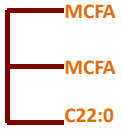
Physical properties = f(composition of FA's esterified)

It has low flash point : cannot be used as frying fat

It doesn't mold well

## Example of Specialty Fats? ...(3)

Caprenin® (Procter & Gamble Co., Cincinnati, OH)




- Randomly structured lipid containing behenic acid (C22:0) obtained from hydrogenated rapeseed oil and two medium-chain fatty acid (caprylic and capric) from Coconut Oil (CO) and Palm Kernel Oil (PKO)
- Low-calorie fats → calorie content : 4 kcal/g
- Originally created to mimic cocoa butter



## Example of Specialty Fats? ...(4)a

Betapol® - a targeted structured lipid (Unilever)

Structured lipid having similar fatty acids distribution with human milk fat

Produced by acydolysis of tripalmitin with unsaturated FA (to give TAG with up to 60% of palmitic acid at the sn-2 position)

Improved fat and calcium absorption (increase production of energy and improved bone mineral density in infant) most likely to be used in infant formula

## Example of Specialty Fats? ...(4)b ...notes

Betapol® - *as Infant Formula*

- Palmitic acid is the most abundant saturated FA in human milk
  - About 70% of palmitic acid in human milk fat are at the sn-2 position
  - > 80% of palmitic acid in vegetable oil are at sn-1 and sn-3 position
    - Palmitic acid in vegetable oil-based formula is hydrolyzed from the glycerol backbone by pancreatic lipase :
      - Free palmitic acid may form insoluble calcium soap and excreted with the feces
      - Unnecessary loss of dietary energy and calcium

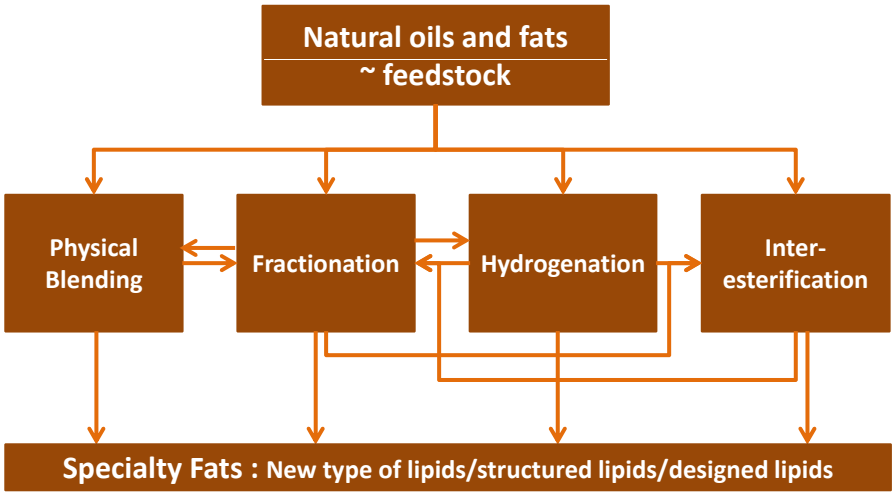


## Example of Specialty Fats? ...(5)

Laurical™ (Calgene Inc., Davis, CA)

- Laurical is a targeted structured lipid created by genetic modification to produce high-laurate canola
- Canola oil normally does not contain lauric acid
- Bioengineered canola containing 40% lauric acid is now available >> Laurical™

## Type of (Bio)technology of Specialty Fats?

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Other data from our own lab


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